Low Phytic Acid Barley Improves Performance, Bone Mineralization, and Phosphorus Retention in Turkey Poults[1]

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Summary

A study was conducted to determine whether P in a low phytic acid mutant barley (*Hordeum vulgare* L.; MB) containing the *lpa 1-1* allele is more available than P in a near-isogenic, wild-type barley (NB). The MB contained 0.21% non-phytate P (nP) (estimated available P; aP) and 0.35% total P (tP), whereas NB contained 0.11% aP and 0.35% tP. A completely randomized design was used with 150 1-d-old male poults randomly assigned to five treatments (six pens of five poults per treatment) for 21 d. The five treatments were 1) a NB diet containing 0.30% aP, 0.41% tP, and 1.0% Ca; 2) a MB diet containing 0.36% aP, 0.41% tP, and 1.0% Ca; 3) a NB diet similar to Diet 1 but with KH_2PO_4 added to increase the aP to 0.36% (0.47% tP) to match the aP in Diet 2; 4) a MB diet containing 0.60% aP, 0.86% tP, and 1.2% Ca; and 5) a NB diet containing 0.60% aP, 0.92% tP, and 1.20% Ca. Performance and bone ash were significantly lower ($P < 0.05$) in poults fed Diet 1 compared with those in poults fed Diet 2. Performance and bone ash were similar ($P > 0.05$) in poults fed Diets 2 and 3 and in poults fed Diets 4 and 5. Poults fed Diet 1 retained 13.9% more P than did poults fed Diet 2 ($P < 0.05$). Poults fed Diets 2 and 4 retained 11.9 and 4.9% more P than poults fed Diets 3 and 5, respectively ($P < 0.05$). Poults fed MB diets excreted 41% less P than did poults fed NB diets when barley was the sole source of phytic acid in the diet. Results of the current study indicate that P in MB is more available than P in NB, and decreasing the phytate content did not compromise the nutritional value of MB.

Key words: Bioavailability, phosphorus, low phytic acid, barley, poults

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Description of Problem

About two-thirds of the P in cereal grains (CG) and oilseed meals (OSM) is bound in the form of phytate and is poorly available to poultry. As a result, rations based on CG and OSM need to be supplemented with inorganic P. One result of the poor availability of phytate P is that a significant amount of P is excreted annually in poultry manure in the US [1]. Poultry manure is usually disposed of by applying it to pastures and cropland. However, in areas of intensive poultry production, the amount applied exceeds the requirement for plant growth. When this occurs, P can pose a significant environmental problem. Increased pressure to reduce environmental pollution from P in animal wastes has stimulated research in ways of utilizing phytate P.

One approach to increase the availability of P in CG and OSM is to genetically manipulate the form of P in these feed ingredients. Recently, scientists developed corn mutants that show reductions in phytate P ranging from 50 to 60% with no reduction in tP [2]. In a chick study, P availability from the low phytic acid corn was found to be 65% compared with 25% from an isogeneic, wild-type corn [3]. A low phytic acid (*lpa*1-1) MB (*Hordeum vulgare* L.) that is phenotypically identical to NB has also been developed by USDA scientists [4]. This low phytic acid barley shows a 45% reduction in phytic acid P with no changes in tP [4]. Previous studies conducted in our laboratory indicated that using toe ash data, P in MB and NB was 49.3 and 28% available, respectively [5]. Increased P availability in MB has also been shown in fish and pigs [6, 7]. The objectives of the present study were to confirm the increased bioavailability of P in MB observed in previous studies and to determine whether MB could replace NB without affecting poult performance. The current study was also designed to evaluate the efficacy of MB in reducing P concentrations in turkey manure.

Materials and Methods

One hundred fifty 1-d-old male turkey poults [8] were purchased from a commercial hatchery, weighed, wing-banded, and randomly assigned to treatments. Poults were maintained on a 24-h constant light schedule and allowed access to feed and water ad libitum. A completely randomized design was used with five dietary treatments and six replicate pens of five poults allotted randomly to each dietary treatment from Days 1 to 21. Dietary treatments included 1) a NB diet containing 0.3% aP, 0.41% tP, and 1.0% Ca; 2) a MB diet containing 0.36% aP, 0.41% tP, and 1.0% Ca; 3) a NB diet similar to Diet 1 but with KH_2PO_4 added to increase the aP to 0.36% (0.47% tP) to match the aP in Diet 2; 4) a MB diet containing 0.60% aP, 0.86% tP, and 1.2% Ca; and 5) a NB diet containing 0.60% aP, 0.92% tP, and 1.20% Ca. Both MB and NB used in the current study were provided by the USDA-Agricultural Research Service [9]. The MB used in the present study was homozygous for the low phytic acid 1-1 (*lpa* 1-1) allele of the low phytic acid 1 gene and was analyzed to contain 0.21% nP and 0.35% total P (tP), whereas NB was analyzed to contain 0.11% nP and 0.35% tP using methods as described by Raboy et al. [10, 11]. In this study, nP was considered as aP. Samples of both MB and NB were analyzed for nutrient contents and amino acid profiles [12].

The composition and selected nutrient composition of the experimental diets are given in Table 1. With the exception of Ca and P in Diets 1, 2, and 3, all diets met or exceeded the nutrient requirements of turkeys [13]. To avoid the confounding effect caused by the presence of nonstarch polysaccharides in barley, all diets were supplemented with 500 g Ronozyme® B (β-glucanase, xylanase, and α-amylase) per ton of diet [14]. In addition, because barley has been shown to contain high endogenous phytase activity, the endogenous phytase activity in both MB and NB was determined [15]. Chromic oxide (0.05%) was also included in the diets as an indigestible marker for determination of P and Ca retention.

Diets 1, 2, and 3 used in the current study were semi-purified-type diets; barley was the only source of phytate. The diets were designed to determine whether MB contains more aP than does NB. Because the only difference between Diets 1 and 2 was the source of barley, differences observed in any response variables should be a result of different aP levels in the diets. Diet 3 was, in fact, Diet 1 supplemented with inorganic P to increase the aP level to match

TABLE 1. Composition and selected nutrient content of experimental diets

INGREDIENT	DIET 1	2	3	4	5
	(%)				
Wild-type barley	60.00	—	60.00	—	50.00
Low phytate barley	—	60.00	—	50.00	—
Soybean meal (48% CP)	—	—	—	29.37	29.37
Casein	18.76	18.76	18.76	—	—
Glucose	8.70	8.70	8.70	—	—
Gelatin	4.80	4.80	4.80	—	—
Limestone	1.90	1.90	1.90	0.55	0.55
Fish meal	1.60	1.60	1.60	5.00	5.00
Pork meal	—	—	—	5.00	5.00
Corn gluten meal	—	—	—	4.96	4.96
Corn oil	0.86	0.86	0.86	3.71	3.71
Potassium carbonate	0.70	0.70	0.70	—	—
Dicalcium phosphate	—	—	—	0.41	0.41
Salt	0.40	0.40	0.40	0.19	0.19
L-Lysine	—	—	—	0.14	0.14
L-Arginine	0.20	0.20	0.20	—	—
DL-Methionine	0.17	0.17	0.17	0.09	0.09
Choline	0.15	0.15	0.15	0.01	0.01
Trace mineral mix[A]	0.11	0.11	0.11	0.11	0.11
Vitamin mix[B]	0.05	0.05	0.05	0.05	0.05
Se mix[C]	0.05	0.05	0.05	0.05	0.05
Chromic oxide	0.05	0.05	0.05	0.05	0.05
Ronozyme® B[D]	0.05	0.05	0.05	0.05	0.05
Celufil	1.45	1.45	1.19	0.26	—
KH_2PO_4	—	—	0.26	—	0.26
Nutrients					
ME, kcal/kg	2,800	2,800	2,800	2,800	2,800
CP, %	28	28	28	28	28
Ca, %	1.00	1.00	1.00	1.20	1.20
Calculated total P, %	0.41	0.41	0.47	0.86	0.92
Analyzed total P, %	0.40	0.38	0.44	0.90	0.94
Estimated available P, %	0.30	0.36	0.36	0.60	0.60

[A]Mineral mix provided (mg/kg diet): MnO_2, 222; ZnO, 209; $FeSO_4 \cdot 7H_2O$, 654; $CuSO_4$, 32; ethylenediamine dihydroiodide, 1.9; and $CaCO_3$, 160.
[B]Supplied(/kg feed): vitamin A (retinyl acetate), 8,810 IU; cholecalciferol, 3,855 IU; vitamin E (dl-α-tocopheryl acetate), 14 IU; niacin, 55 mg; calcium pantothenate, 17 mg; riboflavin, 6.6 mg; pyridoxine, 2.2 mg; menadione sodium bisulfite, 1.7 mg; folic acid, 1.4 mg; thiamin mononitrate, 1.1 mg; biotin, 0.2 mg; cyanocobalamin, 11 μg; and ethoxyquin, 83 mg.
[C]Supplied 0.3 mg Se/kg feed.
[D]Hoffmann-La Roche Inc., Nutley, NJ 07110-1199.

that in Diet 2, so that the only difference in these two diets was the level of phytic acid. Diet 3 was then compared with Diet 2 to determine whether the decreased phytate P content in MB would affect the nutritional value of MB. In addition, because Diets 1, 2, and 3 were semi-purified diets and contained levels of aP lower than the NRC requirement [13], Diets 4 and 5, which were commercial-type diets, were also included in the current study. These two diets were formulated to contain optimal levels of aP; the MB provided a greater proportion of aP (Diet 4) compared with NB (Diet 5). These two diets were designed to determine whether MB could replace NB without affecting poult performance.

During the third week, excreta samples (six pens per treatment) were collected for 5 consecutive d to determine P and Ca retention. On Day 21, poults were killed by asphyxiation with CO_2, and middle toes from both feet were collected from each poult to determine toe ash. The right tibiae (three poults per pen) were also collected to determine tibia ash. Excreta was dried in an oven at 50°C for 24 h and ground to pass through a 1-mm sieve. Feed samples were also ground to pass through a 1-mm sieve. Duplicate samples of excreta and feed were digested by nitric-perchloric acid wet digestion, and the assay was validated by including standard reference material (peach leaves) from the National Institute of Standards and Technology [16]. Phosphorus concentrations in feed were determined colorimetrically by the molybdo-vanadate method [17]. Calcium and Cr were analyzed by flame atomic absorption spectrophotometry. Phosphorus and Ca retention were calculated using the following formula: 100% – [100% × (Cr concentration in feed ÷ Cr concentration in excreta) × (P or Ca concentration in excreta ÷ P or Ca concentration in feed)]. The animal care and use protocol was reviewed and approved by the University of Missouri-Columbia Animal Care and Use Committee.

All data were analyzed using the General Linear Models procedure of SAS® software [18]. Pen was used as the experimental unit. Dietary treatments were compared by single degree of freedom contrasts. Statistical significance was accepted at $P < 0.05$.

Results and Discussion

The nutrient compositions of both MB and NB are listed in Table 2. The analytical results indicated that both MB and NB had similar nutrient contents. With the exception of nP and phytate P values, MB and NB had similar CP, crude fat, crude fiber, amino acid profiles, and selected mineral contents, which suggests that diets containing MB and NB should have similar nutrient values, and differences observed in any response variables should result from the increased nP in MB. Results also showed that the endogenous phytase activity in MB (251 ± 46 U/kg) was similar to that of NB (290 ± 18 U/kg), suggesting that the increased P availability in MB was not due to differences in endogenous phytase activity. The effects of dietary treatments on growth performance are presented in Table 3. Compared with poults fed Diet 1 containing NB, poults fed Diet 2 containing MB consumed 21% more feed, gained 30% more weight, and were more efficient (1.37 vs. 1.56; g:g) in converting feed to gain ($P < 0.05$). Poults fed Diet 2 had similar ($P > 0.05$) feed intake, weight gain, and feed efficiency compared with poults fed Diet 3 containing NB supplemented with KH_2PO_4. Results also showed that feed intake, body weight gain, and feed conversion of poults fed the commercial-type diet containing MB (Diet 4) were not significantly different ($P > 0.05$) from those of poults fed the commercial-type diet containing NB plus KH_2PO_4 (Diet 5).

The effects of dietary treatments on bone mineralization are presented in Table 4. Compared with poults fed Diet 1, poults fed Diet 2 had a higher percentage tibia and toe ash ($P <$

TABLE 2. Analytical nutrient content of low phytate and wild-type barley

	Low phytate barley	Wild-type barley
ME,[A] kcal/kg	2,640	2,640
CP, %	13.09	13.31
Crude fat, %	2.22	1.86
Crude fiber, %	3.92	2.36
Ash, %	2.26	2.36
Moisture, %	11.51	11.46
Amino acid content, %		
Arginine	0.69	0.65
Cysteine	0.30	0.31
Glycine	0.52	0.51
Histidine	0.30	0.29
Isoleucine	0.47	0.49
Leucine	0.90	0.95
Lysine	0.50	0.47
Methionine	0.22	0.23
Phenylalanine	0.68	0.74
Proline	1.39	1.56
Serine	0.46	0.48
Threonine	0.42	0.43
Tryptophan	0.14	0.13
Tyrosine	0.31	0.35
Valine	0.65	0.66
Macro and micro mineral content		
Ca, %	0.051	0.052
K, %	0.42	0.36
Mg, %	0.1	0.1
Fe, mg/kg	55.85	46.85
Mn, mg/kg	15.10	16.70
Cu, mg/kg	12.90	3.15
Zn, mg/kg	27.75	23.10
Analyzed P content, %		
Phytate P	0.14	0.24
Nonphytate P	0.21	0.11
Total P	0.35	0.35

[A]Estimated values.

TABLE 3. Performance of poults fed diets containing wild-type (NB) and low phytate barley (MB)[A]

DIET	BARLEY SOURCE	aP[B]	tP[B]	FEED INTAKE[C]	WEIGHT GAIN[C]	FEED:GAIN[C]
		(%)		(g)		(g:g)
1	NB	0.30	0.41	379	244	1.56
2	MB	0.36	0.41	480	351	1.37
3	NB	0.36	0.47	464	330	1.41
4	MB	0.60	0.86	621	492	1.26
5	NB	0.60	0.92	627	502	1.25
Pooled SEM				13	9	0.03
				P		
1 vs. 2				0.0001	0.0001	0.0001
2 vs. 3				0.3830	0.0992	0.3309
4 vs. 5				0.7556	0.4172	0.6341

[A]Each value represents the mean of six pens of five poults each.
[B]Values in aP (available P) and tP (total P) columns are estimated values.
[D]Overall F test for feed intake, weight gain, and feed:gain was significant ($P < 0.0001$).

0.05). Poults fed Diet 2 had similar percentage tibia and toe ash compared with that of poults fed Diet 3 ($P > 0.05$). There was no significant difference in percentage tibia and toe ash between poults fed Diets 4 and 5 ($P > 0.05$). The effects of dietary treatments on P retention are presented in Table 5. Poults fed Diet 2 had significantly higher P retention than poults fed Diet 1 ($P < 0.05$). Poults fed Diet 2 also had significantly higher P retention compared with poults fed Diet 3 ($P < 0.05$). Compared with poults fed Diet 5, P retention of poults fed Diet 4 was significantly higher ($P < 0.05$). Results of Ca retention are also presented in Table 5. Poults fed Diet 2 had similar Ca retention to poults fed Diets 1 and 3 ($P > 0.05$). Calcium retention in poults fed Diet 4 was not significantly different from that of poults fed Diet 5 ($P > 0.05$).

One of the objectives of the current study was to confirm the increased P availability in MB. Diets 1, 2, and 3 were designed to address this objective. Results showed that growth performance and bone mineralization of poults fed the semi-purified diet containing MB (Diet 2) were improved as compared with those of poults fed the semi-purified diet containing NB (Diet 1). The only differences between these two diets were the source of barley and the increased

TABLE 4. Tibia and toe ash of poults fed diets containing wild-type (NB) and low phytate barley (MB)

DIET	BARLEY SOURCE	aP[A]	tP[A]	TIBIA ASH[B,D]	TOE ASH[C,D]
		(%)			
1	NB	0.30	0.41	25.4	7.31
2	MB	0.36	0.41	30.5	9.03
3	NB	0.36	0.47	29.8	9.00
4	MB	0.60	0.86	44.2	14.39
5	NB	0.60	0.92	44.5	14.12
Pooled SEM				0.4	0.14
				P	
1 vs. 2				0.0001	0.0001
2 vs. 3				0.8777	0.2113
4 vs. 5				0.1647	0.5296

[A]Values in aP (available P) and tP (total P) columns are estimated values.
[B]Each value represents the mean of six pens of three poults each.
[C]Each value represents the mean of six pens of five poults each.
[D]Overall F test for tibia and toe ash was significant ($P < 0.0001$).

TABLE 5. Phosphorus and Ca retention of poults fed diets containing wild-type (NB) and low phytate barley (MB)

DIET	BARLEY SOURCE	aP[A]	tP[A]	Ca	P RETENTION[B,C,D]	Ca RETENTION[B,D,E]
		(%)				
1	NB	0.30	0.41	1.0	63.0	38.4
2	MB	0.36	0.41	1.0	76.4	41.2
3	NB	0.36	0.47	1.0	64.5	43.5
4	MB	0.60	0.86	1.2	50.5	51.3
5	NB	0.60	0.92	1.2	45.6	50.9
Pooled SEM					0.8	1.3
					P	
1 vs. 2					0.0001	0.1475
2 vs. 3					0.0001	0.2276
4 vs. 5					0.0004	0.8566

[A]Values in aP (available P) and tP (total P) columns are estimated values.
[B]Each value represents the mean of six pens of five poults each.
[C]Percentage P retention = 100% − [100% × (Cr concentration in feed ÷ Cr concentration in excreta) × (P concentration in excreta ÷ P concentration in feed)].
[D]Overall F test was significant ($P < 0.0001$).
[E]Percentage Ca retention = 100% − [100% × (Cr concentration in feed ÷ Cr concentration in excreta) × (Ca concentration in excreta ÷ Ca concentration in feed)].

amount of aP present in Diet 2 because of the use of MB (Diet 1, 0.3% aP; Diet 2, 0.36% aP). Better performance and bone mineralization observed in poults fed Diet 2 indicate that P in MB is more available than P in NB. These results suggest that a change in a single gene in MB has a positive effect on the nutritional quality of the grain, which results in increased animal productivity. In addition, a semi-purified diet containing NB supplemented with KH_2PO_4 (Diet 3) was formulated to contain 0.36% aP to match the level of aP in Diet 2. Results showed that poults fed Diets 2 and 3 had similar performance and bone mineralization. These data indicate that the estimated value of aP in MB is reasonable and that lowering the phytate content in MB did not result in any detectable differences in the nutritional value of MB.

Because Diets 1, 2, and 3 were semi-purified diets and the aP level in these diets was lower than the NRC requirement, Diets 4 and 5 were formulated to contain the optimal level of aP in a commercial-type diet to determine whether substituting MB in commercial-type diets would affect poult performance. Results showed that poults fed the commercial-type diet containing MB (Diet 4) had similar performance and bone mineralization compared with poults fed the commercial-type diet containing NB supplemented with KH_2PO_4 (Diet 5), indicating that MB had the same nutritional value as NB, and replacing NB with MB in the diet did not affect poult performance. Similar results were also observed in previous studies conducted in fish and swine [6, 7, 19].

Results of the current study also indicated that poults fed diets containing MB had significantly higher P retention than did poults fed diets containing NB. Poults fed Diet 2, which contained MB, retained 12 and 11% more P compared with poults fed Diets 1 and 3, which contained NB. This, again, indicates higher P availability in MB compared with NB. In addition, when MB was substituted for NB in a commercial-type diet, P retention was increased by 5%. The current study also showed that excreta P concentration was reduced 41% by MB compared with NB when barley was the sole source of phytic acid in the diet (excreta P with MB, 0.38%; excreta P with NB, 0.65%). When MB was substituted for NB in a commercial-type diet, there was a 17% reduction in P excretion (excreta P with MB, 1.28%; excreta P with NB, 1.55%). Lower P excretion for diets containing MB compared with NB was also observed in other species. In swine, Veum et al. [19] reported that when barley was the sole source of phytic acid in the diet, P excretion was reduced by 55% in growing pigs by substituting MB for NB. When a commercial-type diet was given to growing pigs, a 15% reduction in excreta P was observed in pigs fed MB compared with pigs fed NB. Sugiura et al. [6] observed a 44% reduction in tP excretion by replacing NB with MB in fish diet. Sugiura et al. [6] also suggested that the difference in P availability between MB and NB should become more evident by reducing the P concentration in the diet to the minimum levels required by fish.

Results of the current study showed that poults fed MB and NB had similar Ca retention, which is consistent with the findings of Sugiura et al. [6]. However, these results are in contrast to the findings of Veum et al. [19] who reported that Ca retention in pigs fed a MB diet was higher than that in pigs fed a NB diet when barley was the only source of phytate in the diets. Phytate is known as a strong chelating agent that interacts with divalent cations, such as Ca, rendering it unavailable for absorption. Failure to demonstrate an increase in Ca retention in poults fed diets containing MB in the present study could be due to species differences or due to the fact that the level of Ca used in the current study was not low enough to pick up differences in Ca retention.

In summary, results of the present study clearly demonstrated that P in MB was more available than P in NB, and reducing the phytate content in MB did not compromise its nutritional value. Results also showed that poults fed diets containing MB had higher P retention than poults fed diets containing NB, suggesting that, when poultry are fed barley-based feeds, the amount of P excreted in poultry waste should be substantially reduced by substituting MB for NB.

Conclusions and Applications

1. Data from the current study confirm the increased P availability in MB observed in previous studies. As a consequence of the increased P availability in MB, diets containing low phytic acid barley will not need to be supplemented with as much inorganic P.
2. The lower phytate content in MB did not compromise the nutritional value of MB, indicating that MB can replace normal barley in turkey rations without affecting performance.
3. Increased P availability in MB should substantially reduce P excretion in poultry manure; therefore, the environmental consequences of land application of poultry litter should be significantly decreased. Low phytate barley combined with other low phytate grains provides an alternative tool to improve P utilization and minimize the impact of poultry manure P on the environment.

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15. A 1-g sample of grain was mixed with 20 mL of 0.2*M* citrate buffer (pH 5.5) in 1*M* NaCl solution and extracted on a magnetic stirrer at 30°C for 30 min. The slurry was centrifuged, and the clear supernatant was cooled on an ice bath and served as a source of enzyme. One milliliter of enzyme solution was pipetted into 1 mL of 5 m*M* sodium phytate solution and incubated for 120 min. The reaction was stopped by the addition of 2 mL 15% TCA. The concentration of P was determined by the standard Fiske Subbarow method. One unit of phytase activity was defined as the amount of enzyme that frees 1 μmol inorganic P from 1.5 m*M*-sodium phytate/min at pH 5.5 and 37°C.

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